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Influence of Sex and Age on the Normal Range of Eleven Serum Constituents[1)]

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Serum concentrations of calcium, inorganic phosphorus, total protein, albumin, urea nitrogen, uric acid, cholesterol, total bilirubin, alkaline phosphatase (EC 3.1.3.1), lactate dehydrogenase (EC 1.1.1.27) and serum aspartate transaminase (EC 2.6.1.1) were measured in a large ambulatory population to provide normal values according to sex and age. Significant differences are apparently related to the combined effects of ageing and differences in the activity of sex hormones. Puberty and adolescence in both sexes and menopause in females are the periods of life when the most marked changes occur.
Shifts in serum calcium correlate with those of total protein and albumin and may represent a secondary effect. The product of serum calcium and phosphorus concentration differs between sexes and changes with age. It is mostly determined by the serum phosphorus concentration, which in turn may be a function of the changing activity of alkaline phosphatase.

Die Serumkonzentrationen von Calcium, anorganischem Phosphor, Gesamteiweiß, Albumin, Harnstoff-N, Harnsäure, Cholesterin, Gesamtbilirubin, Alkalischer Phosphatase (EC 3.1.3.1), Lactatdehydrogenase (EC 1.1.1.27) und Serum-Aspartattransaminase (EC 2.6.1.1) wurden bei einer großen Anzahl ambulanter Probanden bestimmt, um Normalwerte bezüglich Geschlecht und Alter zu erhalten. Signifikante Unterschiede sind offenbar abhängig vom Zusammenwirken von Altern und Aktivitätsunterschieden der Sexualhormone. Die bedeutendsten Veränderungen findet man bei beiden Geschlechtern während Pubertät und Adoleszenz und bei Frauen in der Menopause.
Änderungen der Calciumkonzentration korrelieren mit solchen der Gesamteiweiß- und Albuminkonzentration, sie mögen sekundärer Natur sein. Das Produkt von Calcium- und Phosphorkonzentration im Serum ist bei den Geschlechtern verschieden und ändert sich mit dem Alter. Es ist hauptsächlich von der Phosphorkonzentration im Serum abhängig, die wiederum durch Änderungen der Aktivität der Alkalischen Phosphatase bedingt sein könnte.

Establishing the range of "normal" values presents two major difficulties. (1) Normal variation reflects multiple genetical and environmental influences causing intra- and inter-individual variability. In particular, normal values vary with sex and age; hence, normal values based on small populations biased to one sex and a particular age group cannot be used for general reference. (2) "Normal" is not readily defined. Conceptually it cannot be equated with ideal or perfect and statistically it is not necessarily well represented by the gaussian distribution (i. e. mean, standard deviation). Both difficulties can be overcome by collecting sufficient data to derive sex-specific and age-specific values and to provide an assessment of "normal" in terms of that which occurs usually or frequently.
This study therefore describes the serum concentrations of calcium, inorganic phosphorus, total protein, albumin, urea nitrogen, uric acid, cholesterol, total bilirubin, alkaline phosphatase, lactate dehydrogenase, and aspartate transaminase in a large ambulatory population.

[1)] Presented at the Technicon International Congress on Automation, Chicago, Ill., August 1969.

Materials and Methods

Bloods specimens were obtained from more than 3000 visitors to the 1968 San Francisco Health Fair. Each subject completed a questionnaire concerning age, sex, time of last meal, general health, and drug medication. Immediately after the blood was drawn the specimens were analyzed on the site with a Technicon SMA 12/60. Methods are listed in Table 1. Quality control included the routine use of assayed controls[2)] to tests accuracy as well as commercial unassayed serum pools[2)] to test precision. Since the method errors vary with concentration, Table 2 lists quality control data of two control sera of different composition, control A having mostly low, control B high concentrations. After eliminating results from subjects with a history of active disease or use of drugs, the data were grouped by sex and age in decades. (The first two decades were divided at age 13 in an attempt to separate pre- and post-puberty subjects.)
Calcium was determined by atomic absorption spectroscopy with a Perkin-Elmer 303 instrument[3)] with a three-slot Boling burner and a digital readout unit (model DCR 1) which automatically computes the average of four independent observations for each sample. The instrument settings recommended by the manufacturer were used (1). Serum was diluted with the following solution: lanthanum oxide 12.0 gm, sodium chloride 18.0 gm, concentrated

[2)] Hyland control sera, Hyland Laboratories, Los Angeles, California.
[3)] Perkin-Elmer Corporation, Norwalk, Conn.

hydrochloric acid 30 m*l*, Sterox 2 m*l*, deionized water to make 2000 m*l*. At a 1:20 dilution signal response was consistently linear up to at least 16 mg/100 m*l* calcium. Each specimen was analyzed in duplicate, and a standard was run between unknowns. For more than one year the coefficient of variation of this method never exceeded 3% in monthly control periods.

Tab. 1
Methods used on the Technicon SMA 12/60

Constituent	Method	Reference
Calcium	komplexometric (8-hydroxy-quinoline)	H. J. GITELMAN Analytic. Biochem. *18*, 521 (1967)
Inorganic Phosphorus	phosphomolybdic acid, stannous chloride-hydrazine	M. KRAML Clin. Chim. Acta Amsterdam *13*, 442 (1966)
Total Protein	Biuret	J. F. FAILING, M. W. BUCKLEY and B. ZAK Amer. J. Clin. Path. *33*, 83 (1960)
Albumin	dye binding (HABA)	H. H. NISHI and A. RHODES Automation in Analytical Chemistry, Proceedings of the 1965 Technicon Symposium
Urea Nitrogen	diacetyl monoxime	W. H. MARSH, B. FINGERHUT and H. MILLER Clin. Chem. New York *11*, 624 (1965)
Uric Acid	phosphotungstate and hydroxylamine	H. H. NISHI Clin. Chem. New York *13*, 12 (1967)
Cholesterol	acetic acid, acetic anhydride and sulfuric acid	T. C. HUANG, C. P. CHEN, W. WEFLER and A. RAFTERY Analytic. Chem. *33*, 1405 (1961)
Total Bilirubin	azobilirubin	S. R. GAMBINO and H. SCHREIBER Amer. Soc. Clin. Path., Check Sample CC 48, 1968
Alkaline Phosphatase	*p*-nitrophenyl-phosphate, 2-amino-2-methyl-1-propanol	S. MORGENSTERN, G. KESSLER, J. AUERBACH, R. V. FLOR and B. KLEIN Clin. Chem. New York *11*, 876 (1965)
Lactate Dehydrogenase	oxidation of L-lactate by NAD coupled to diaphorase and tetrazolium dye (INT)	N. J. HOCHELLA and S. WEINHOUSE Analytic. Biochem. *13*, 322 (1965)
Aspartate Transaminase	oxalacetic acid is reacted with Azoene Fast Red (PDC)	S. MORGENSTERN, M. OKLANDER, J. AUERBACH, J. KAUFMAN and B. KLEIN Clin. Chem. New York *12*, 95 (1966)

Tab. 2
Precision of assay. Two control sera of different composition were analyzed throughout the entire series of determinations. Number of assays (n), means ($\bar{x}$) and standard deviations (S. D.) are listed

Constituent	Control A			Control B		
	n	$\bar{x}$	S. D.	n	$\bar{x}$	S. D.
Calcium (mg/100 m*l*)	55	8.69	.20	115	11.7	.26
Inorganic Phosphorus (mg/100 m*l*)	56	3.49	.06	116	8.21	.20
Total Protein (g/100 m*l*)	57	6.22	.11	118	5.24	.11
Albumin (g/100 m*l*)	57	3.54	.07	118	2.57	.08
Urea Nitrogen (mg/100 m*l*)	57	11.4	.76	118	52.9	3.03
Uric Acid (mg/100 m*l*)	57	5.04	.25	116	8.89	.43
Cholesterol (mg/100 m*l*)	50	191	10.4	111	193	9.1
Total Bilirubin (mg/100 m*l*)	57	.32	.06	115	6.82	.34
Alkaline Phosphatase (U/*l*)	53	34.5	1.8	115	93.4	3.5
Lactate Dehydrogenase (U/*l*)	57	106	5.1	114	242	10.9
Aspartate Transaminase (U/*l*)	53	39.4	5.5	113	83.5	5.8

Results

The reliability of calcium determination on the Technicon SMA 12/60 was checked by comparing 192 specimes with atomic absorption spectroscopy. Means and ranges obtained with the two methods are given in Table 3.

Tab. 3
Comparison of serum calcium concentrations (mg/100 m*l*)

Method	Mean	Range (mean ± 2 S. D.)
Atomic Absorption Spectroscopy	10.08	9.38 — 10.77
Technicon, SMA 12/60	9.99	9.00 — 10.97

Results obtained with the SMA 12/60 showed somewhat greater scatter than those from atomic absorption spectroscopy (coefficient of correlation, $r = 0.7011$). When the results of atomic absorption spectroscopy were used as an independent variable (X), and the SMA 12/60 results as a dependent variable (Y) the regression equation was: $Y = 0.014 + 0.990\ X$.

When grouped according to sex and age, results of calcium, inorganic phosphorus, total protein and albumin concentrations were normally (gaussian) distributed, plotting as straight lines on probability grids. Means and standard deviations, therefore, adequately describe results (Figs. 1 to 4). The findings reported in these figures (as those reported in the Figures 8 to 14), and in Tables 4 and 5 showing the significance of sex differences and age shifts, were based only on the subjects remaining after persons taking drug medication or having a previously known disease were excluded. To demonstrate the similarity of this "healthy" group with the entire population Table 6 lists calcium, inorganic phosphorus, total protein and albumin concentrations of females and males in the 30 to 39 years and 60 to 69 years age groups as examples. Addition of subjects taking drug medication or having a previously known disease to the "healthy" group hardly affected mean values, nor did this significantly increase the scatter of results (standard deviations) except in isolated instances.

In males serum *calcium* increased after sexual maturation and fell again after the third decade, while in females it decreased during the reproductive age, rose again after the fourth decade, and finally fell after the sixth decade. The variability is similar for sex and age (Fig. 1).

Inorganic phosphorus fell markedly in the first two decades in both sexes, later decreasing less in males and rising again after the fifth decade in females. Variability was highest during childhood and adolescence in both sexes and during the menopausal age in females, when pronounced shifts of concentration occurred (Fig. 2).

Total protein increased until puberty in both sexes. In males it continued to rise until the fourth decade, falling later, while in females it decreased slightly from the second to the sixth decade, and decreased more markedly later (Fig. 3).

Tab. 4

Sex differences of serum calcium, phosphorus, total protein, and albumin concentrations. Means and significance of their differences are listed

Age Group (years)	Calcium (mg/100 ml) ♀	♂	P	Phosphorus (mg/100 ml) ♀	♂	P	Total Protein (g/100 ml) ♀	♂	P	Albumin (g/100 ml) ♀	♂	P
0—12	10.06	10.08	N. S.	5.07	5.28	< .05	7.25	7.24	N. S.	4.29	4.28	N. S.
13—19	10.08	10.13	N. S.	4.57	4.89	< .01	7.58	7.49	N. S.	4.29	4.60	< .001
20—29	9.99	10.20	< .001	4.00	4.17	< .01	7.54	7.67	< .05	4.21	4.52	< .001
30—39	9.87	10.12	< .001	3.88	4.11	< .001	7.49	7.63	< .05	4.11	4.44	< .001
40—49	9.91	10.03	< .05	3.87	3.97	N. S.	7.48	7.51	N. S.	4.12	4.32	< .001
50—59	10.01	9.92	N. S.	3.98	3.87	N. S.	7.49	7.46	N. S.	4.10	4.23	< .01
60—69	9.96	9.95	N. S.	4.02	3.78	< .001	7.35	7.39	N. S.	4.07	4.08	N. S.
70—79	9.82	9.83	N. S.	4.09	3.78	< .01	7.36	7.51	N. S.	4.03	4.03	N. S.

P = probability of significance N. S. = not significant

Tab. 4

Sex differences of serum urea nitrogen, uric acid, cholesterol, and total bilirubin concentrations. Means and significance of their differences are listed

Age Group (years)	Urea Nitrogen (mg/100 ml) ♀	♂	P	Uric Acid (mg/100 ml) ♀	♂	P	Cholesterol (mg/100 ml) ♀	♂	P	Total Bilirubin (mg/100 ml) ♀	♂	P
0—12	15.1	17.5	< .001	4.21	4.40	N. S.	197	194	N. S.	0.43	0.39	N. S.
13—19	14.7	16.3	< .05	4.79	5.82	< .001	198	197	N. S.	0.61	0.69	N. S.
20—29	13.8	17.5	< .001	4.66	6.23	< .001	224	227	N. S.	0.51	0.72	< .001
30—39	14.2	17.0	< .001	4.69	6.44	< .001	230	242	< .05	0.46	0.65	< .001
40—49	16.3	17.9	< .001	4.97	6.35	< .001	215	246	< .001	0.47	0.62	< .001
50—59	16.9	18.9	< .001	5.26	6.42	< .001	272	254	< .001	0.46	0.61	< .001
60—69	18.9	20.4	< .01	5.43	6.39	< .001	285	259	< .001	0.48	0.55	< .01
70—79	18.9	18.1	N. S.	5.20	6.19	< .01	275	258	< .05	0.48	0.67	< .01

P = probability of significance N. S. = not significant

Tab. 4

Sex differences of serum alkaline phosphatase, lactate dehydrogenase and aspartate transaminase concentrations. Means and significance of their differences are listed

Age Group (years)	Alkaline Phosphatase (U/l) ♀	♂	P	Lactate Dehydrogenase (U/l) ♀	♂	P	Aspartate Transaminase (U/l) ♀	♂	P
0—12	167.7	166.8	N. S.	186	203	< .01	38.4	38.7	N. S.
13—19	71.4	138.8	< .001	146	172	< .001	30.6	34.2	< .05
20—29	41.4	50.1	< .001	138	145	N. S.	29.2	31.6	< .01
30—39	41.9	48.2	< .001	143	153	< .05	29.0	32.9	< .001
40—49	43.2	51.4	< .001	148	148	N. S.	30.4	33.9	< .01
50—59	54.0	55.5	N. S.	161	159	< .01	33.8	34.8	N. S.
60—69	61.7	57.3	N. S.	173	159	< .01	33.7	34.7	N. S.
70—79	60.9	56.8	N. S.	163	176	N. S.	30.4	38.0	N. S.

P = probability of significance N. S. = not significant

Tab. 5

Age shifts of serum calcium, inorganic phosphorus, total protein, albumin, urea nitrogen, and uric acid. Probabilities of significance are listed

Interaction	Calcium	Inorganic Phosphorus	Total Protein	Albumin	Urea Nitrogen	Uric Acid
Females (age groups)						
0—12 vs. 30—39	< .01 ↓	< .001 ↓	< .001 ↑	< .001 ↓	< .05 ↓	< .01 ↑
30—39 vs. 60—69	N. S.	< .01 ↑	< .01 ↓	N. S.	< .001 ↑	< .001 ↑
0—12 vs. 60—69	N. S.	< .001 ↓	N. S.	< .001 ↓	< .001 ↑	< .001 ↑
Males (age groups)						
0—12 vs. 20—29	< .05 ↑	< .001 ↓	< .001 ↑	< .001 ↑	N. S.	< .001 ↑
20—29 vs. 60—69	< .001 ↓	< .001 ↓	< .001 ↓	< .001 ↓	< .001 ↑	N. S.
0—12 vs. 60—69	< .05 ↓	< .001 ↓	N. S.	< .001 ↓	< .001 ↑	< .001 ↑

Arrow indicates direction of shift. N. S. = not significant.

Tab. 5

Age shifts of cholesterol, total bilirubin, alkaline phosphatase, lactate dehydrogenase and aspartate transaminase. Probabilities of significance are listed

Interaction	Cholesterol	Total Bilirubin	Alkaline Phosphatase	Lactate Dehydrogenase	Aspartate Transaminase
Females (age groups)					
0—12 vs. 30—39	< .001 ↑	N. S.	< .001 ↓	< .001 ↓	< .001 ↓
30—39 vs. 60—69	< .001 ↑	N. S.	< .001 ↑	< .001 ↑	< .001 ↑
0—12 vs. 60—69	< .001 ↑	N. S.	< .001 ↓	< .05 ↓	< .001 ↓
Males (age groups)					
0—12 vs. 20—29	< .001 ↑	< .001 ↑	< .001 ↓	< .001 ↓	< .001 ↓
20—29 vs. 60—69	< .001 ↑	< .001 ↓	< .05 ↑	< .01 ↑	N. S.
0—12 vs. 60—69	< .001 ↑	< .001 ↑	< .001 ↓	< .001 ↓	N. S.

Arrow indicates direction of shift. N. S. = not significant.

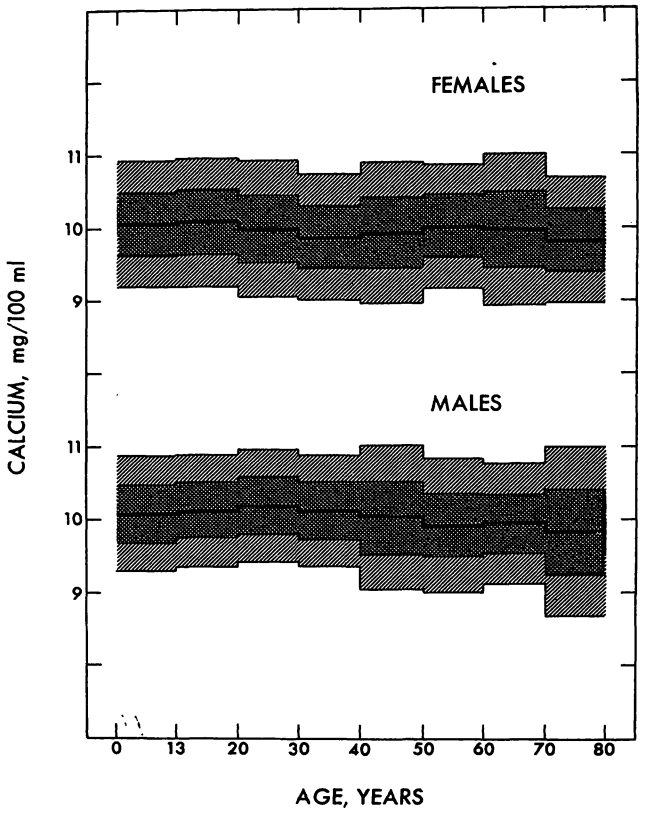

Fig. 1
Serum calcium concentrations grouped according to sex and age. Means are indicated by the heavy line, ± 1 standard deviations are dark gray, ± 2 standard deviations are light gray

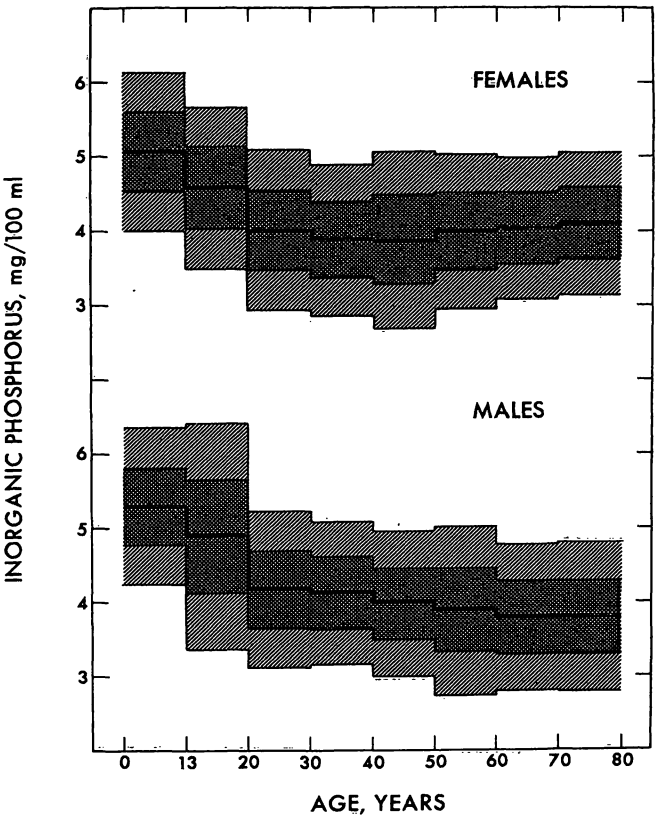

Fig. 2
Serum phosphorus concentrations grouped according to sex and age. Means are indicated by the heavy line, ± 1 standard deviations are dark gray, ± 2 standard deviations are light gray

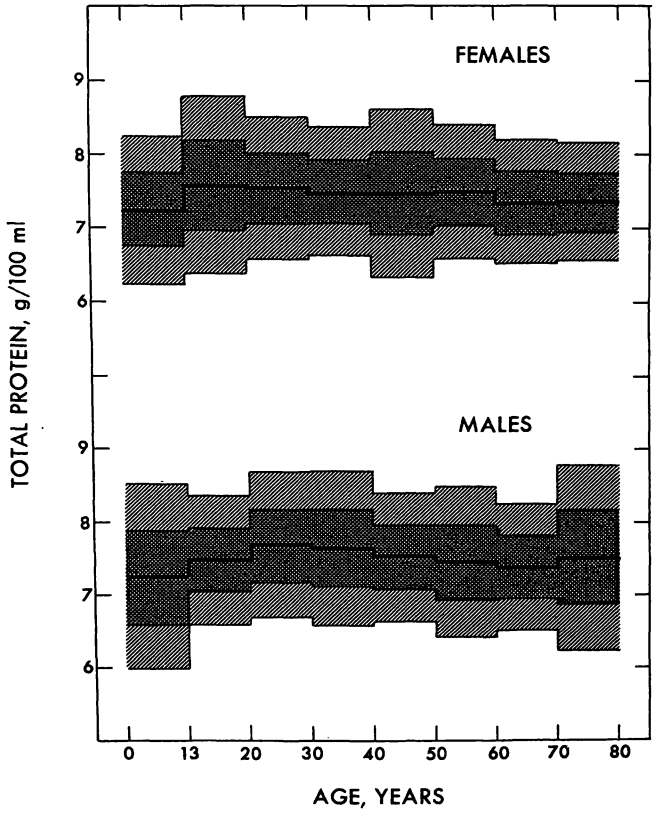

Fig. 3
Serum total protein concentrations grouped according to sex and age. Means are indicated by the heavy line, ± 1 standard deviations are dark gray, ± 2 standard deviations are light gray

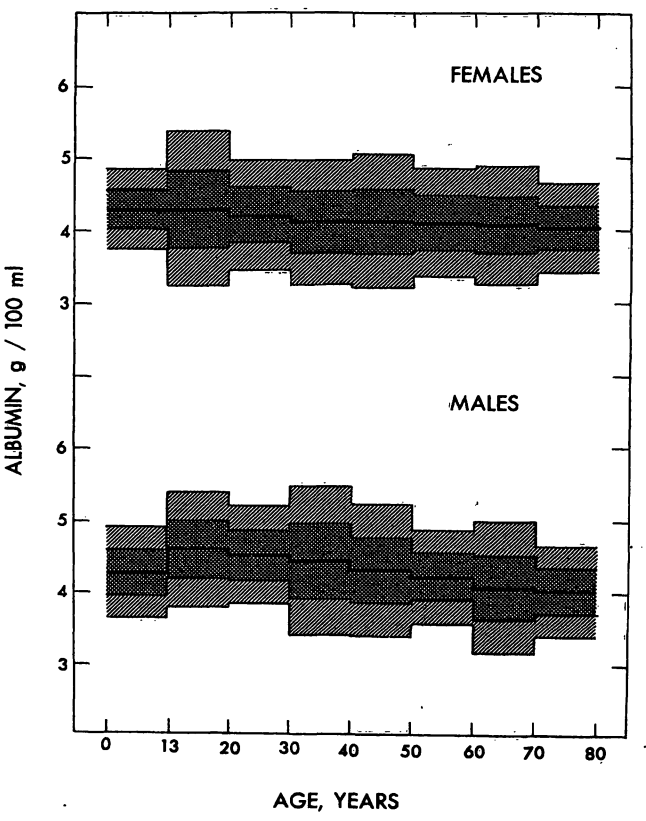

Fig. 4
Serum albumin concentrations grouped according to sex and age. Means are indicated by the heavy line, ± 1 standard deviations are dark gray, ± 2 standard deviations are light gray

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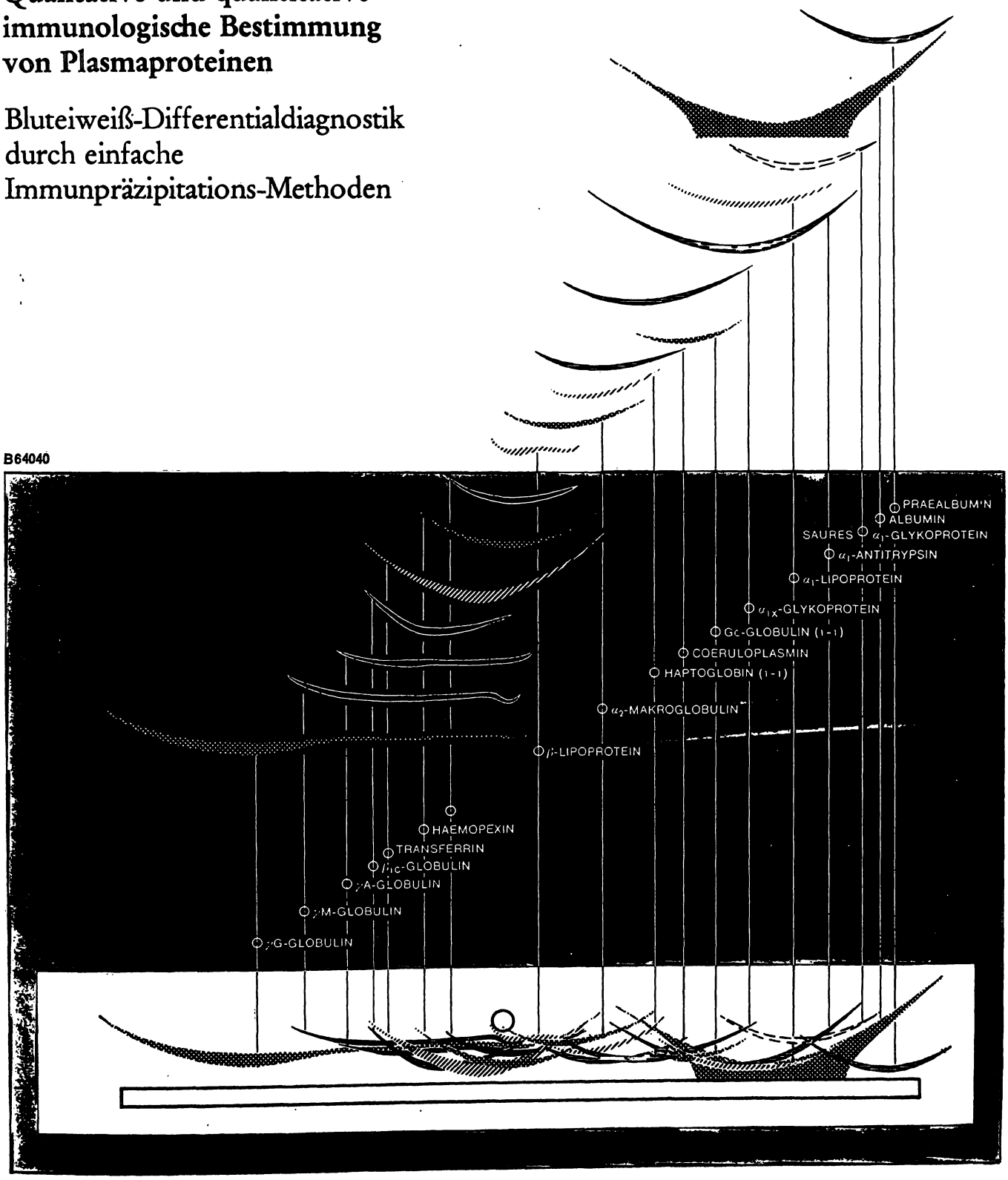

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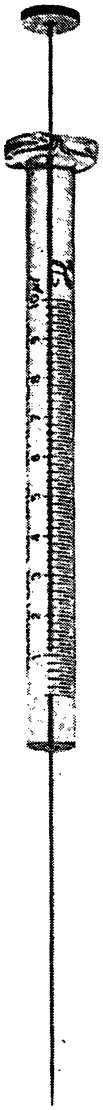

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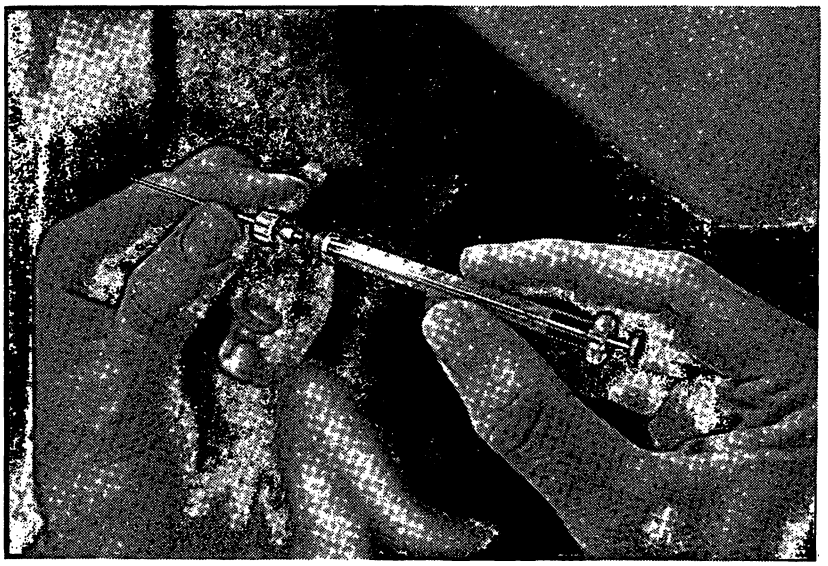

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Tab. 6
Serum calcium, inorganic phosphorus, total protein and albumin concentrations in individuals of given sex-age groups considered "Healthy" (A), and in the entire population of the same groups (B). Number of subjects (n), means and standard deviations are listed

Group		n	Calcium mg/100 ml	Inorganic Phosphorus mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml
Females						
30—39 years	A	173	9.87 ± .43	3.88 ± .51	7.49 ± .42	4.11 ± .33
	B	261	9.87 ± .45	3.88 ± .51	7.47 ± .41	4.11 ± .43
60—69 years	A	134	9.96 ± .51	4.02 ± .48	7.35 ± .42	4.07 ± .40
	B	261	10.01 ± .53	3.99 ± .61	7.36 ± .48	4.06 ± .46
Males						
30—39 years	A	138	10.12 ± .38	4.11 ± .48	7.63 ± .52	4.44 ± .47
	B	171	10.11 ± .39	4.10 ± .51	7.61 ± .54	4.42 ± .52
60—69 years	A	85	9.95 ± .41	3.78 ± .49	7.39 ± .44	4.08 ± .46
	B	146	9.95 ± .43	3.80 ± .52	7.42 ± .44	4.04 ± .53

In females *albumin* fell significantly after the second decade, but the decrease in later life was not significant. In males albumin rose at puberty and decreased thereafter (Fig. 4).

In order to obtain sufficiently large subgroups all subjects were included to study the influence of the *time of last meal* on the four normally distributed parameters (Fig. 5). Although inorganic phosphorus showed the most marked effects, frequently all four parameters shifted concomitantly in the same direction for a given group, suggesting differences in hemoconcentration. Usually values were lowest $^1/_2$ to $2^1/_2$ hours after the last meal, but no uniformly consistent pattern was found.

The highly significant correlations of calcium with total protein and albumin concentrations, derived from the entire population, are shown in Figures 6 and 7. Correlation of body height with calcium, or inorganic phosphorus concentration could not be demonstrated. Correlation coefficients (r) calculated from results of the entire adult population grouped according to sex and age in decades varied between +0.1721 and —0.1452. Table 7 lists the products of serum calcium and in-

Tab. 7
Products of serum calcium and phosphorus concentrations (both in mg/100 ml) according to sex and age

Age Group (years)	♀ $\bar{x}$	± S. D.	♂ $\bar{x}$	± S. D.	P
0—12	50.71	5.54	53.54	6.44	< .01
13—19	46.08	5.85	49.32	7.29	< .01
20—29	39.68	6.46	42.30	5.81	< .001
30—39	38.63	6.26	41.62	5.62	< .001
40—49	38.24	6.29	39.55	6.21	< .05
50—59	38.87	6.12	38.29	6.07	N. S.
60—69	40.05	7.60	37.84	5.70	< .01
70—79	39.74	5.25	36.35	5.35	< .001

P = probability of significance N. S. = not significant

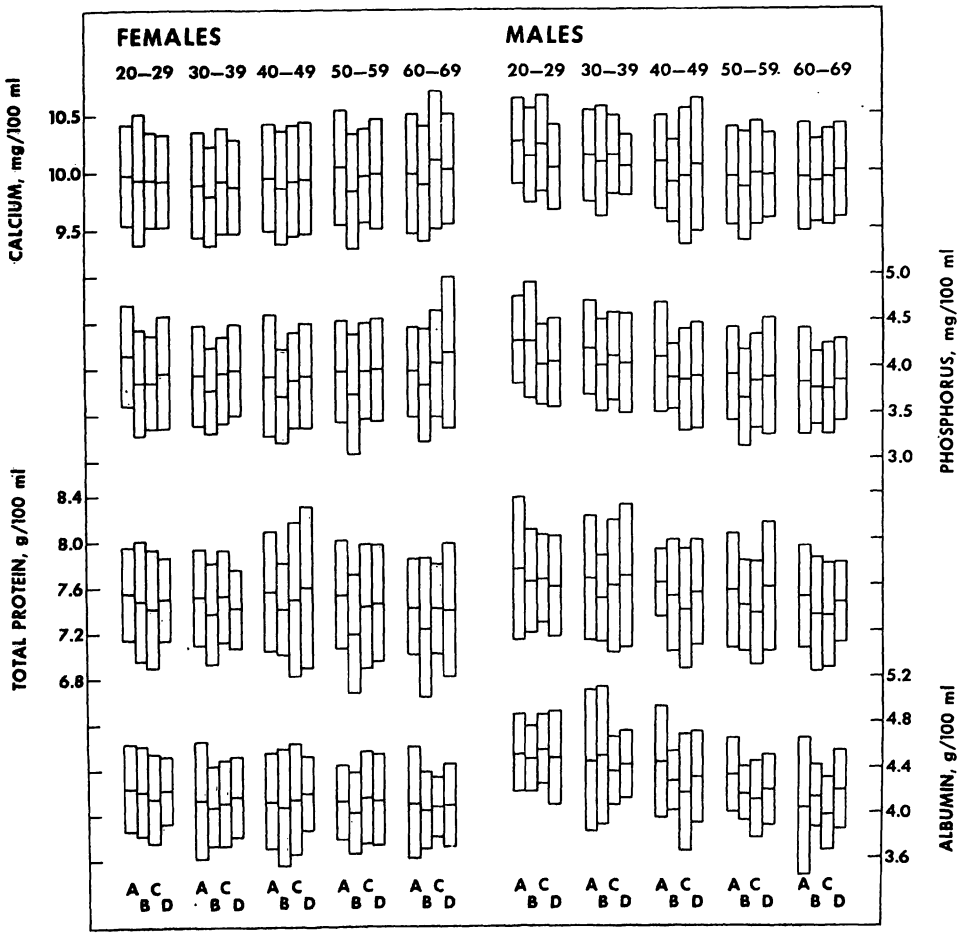

Fig. 5
Serum calcium, phosphorus, total protein and albumin concentrations grouped according to sex, age and time after meal. A = over $3^1/_2$ hours, B = $^1/_2$ to $1^1/_2$ hours, C = $1^1/_2$ to $2^1/_2$ hours, D = $2^1/_2$ to $3^1/_2$ hours. (Means ± 1 S. D.)

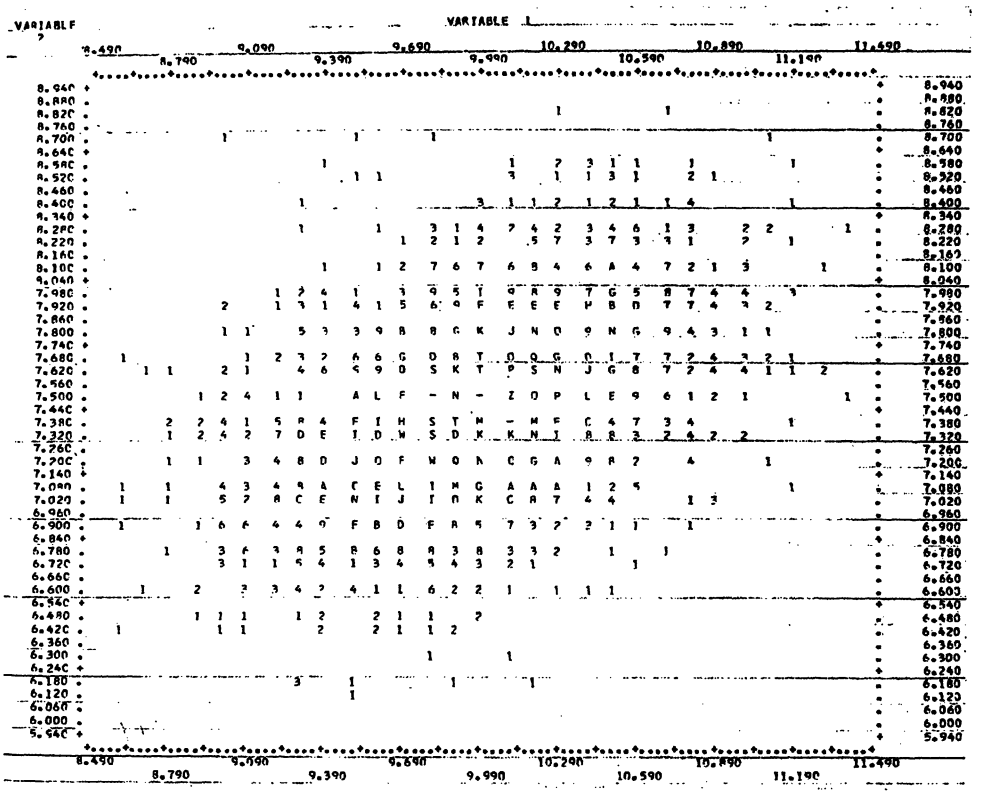

Fig. 6
Correlation of serum calcium (in mg/100 ml, x-axis) and total protein (in g/100 ml, y-axis) of 2891 specimens. Numbers indicate observations at any given value. (A = 10, B = 11, ... Z = 35, — = 36 and more.) Correlation coefficient, r = 0.4589 (P < 0.001). Estimating equation, Ca (mg/100 ml) = 6.255 + 0.4998 total protein (g/100 ml)

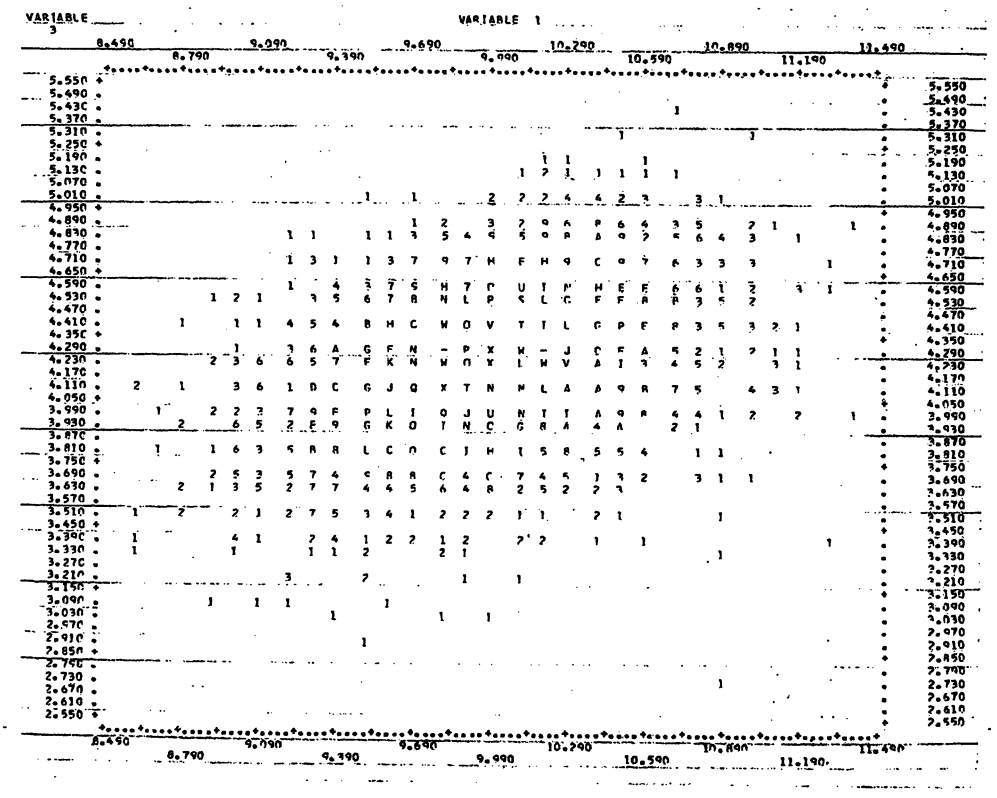

Fig. 7
Correlation of serum calcium (in mg/100 ml, x-axis) and albumin (in g/100 ml, y-axis) of 2891 specimens. Numbers indicate observations at any given value. (A = 10, B = 11, ... Z = 35, — = 36 and more.) Correlation coefficient, r = 0.3859 (P < 0.001). Estimating equation, Ca (mg/100 ml) = 7.946 + 0.4830 albumin (g/100 ml)

organic phosphorus concentration grouped according to sex and age, as well as the significance of sex differences.

Frequency distributions of results from urea nitrogen, uric acid, cholesterol, total bilirubin, alkaline phosphatase, lactate dehydrogenase and aspartate transaminase departed from normal (gaussian) distributions, skewing to high values, and often approximating log-normal distributions reported by others (2—6). Since such data are not adequately characterized by their means and standard deviations, Figures 8 to 14 present results as cumulative percentiles (7). This treatment requires no assumptions about the type of data distribution. The significance of sex and age differences, however, were calcul-

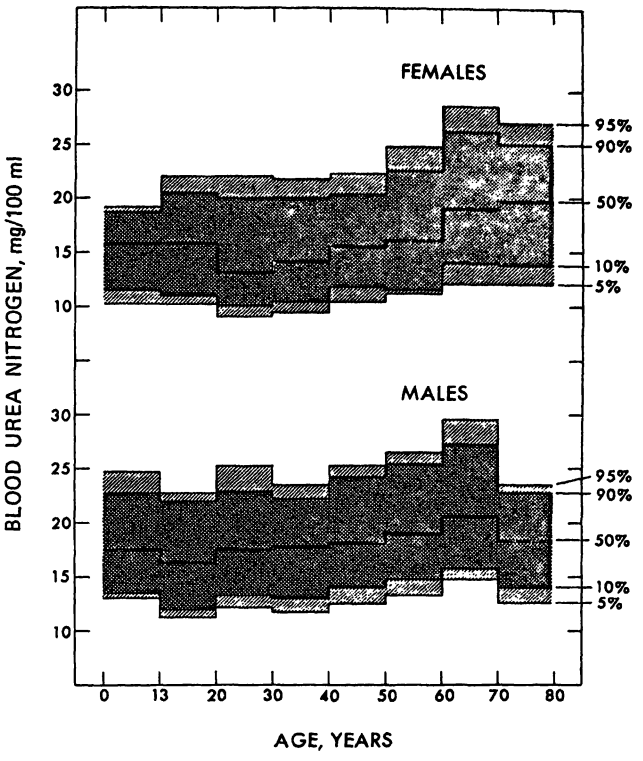

Fig. 8
Serum urea nitrogen concentrations in percentiles grouped according to sex and age. The median (50 percentile) is indicated by the heavy line, 10—90 percentiles dark gray, 5—95 percentiles light gray

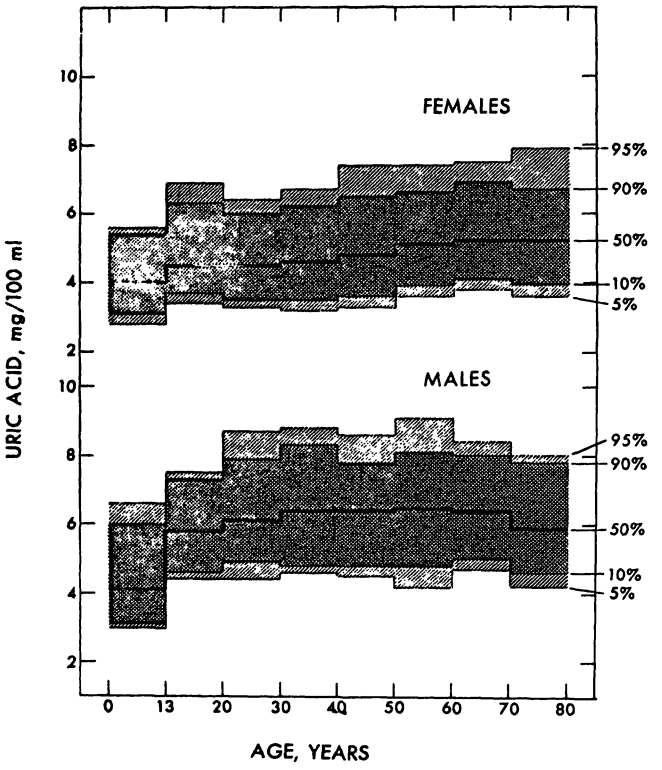

Fig. 9
Serum uric acid concentrations in percentiles grouped according to sex and age. The median (50 percentile) is indicated by the heavy line, 10—90 percentiles dark gray, 5—95 percentiles light gray

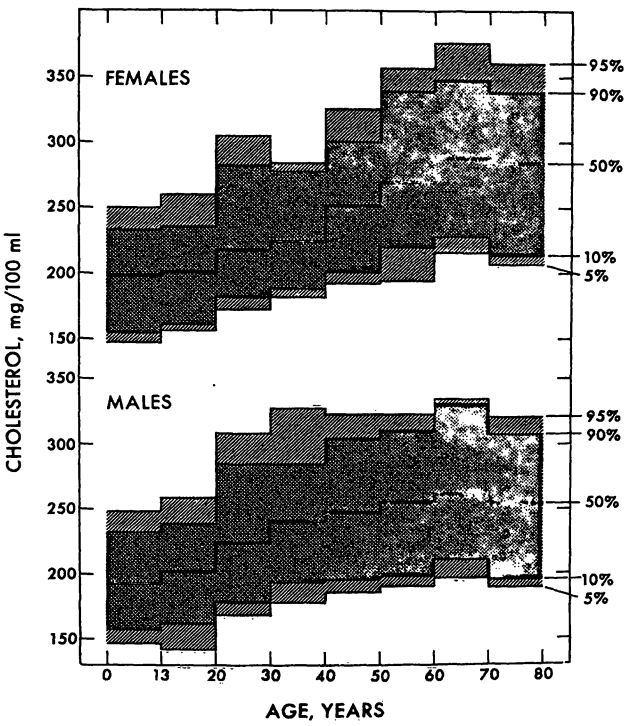

Fig. 10
Serum cholesterol concentrations in percentiles grouped according to sex and age. The median (50 percentile) is indicated by the heavy line, 10—90 percentiles dark gray, 5—95 percentiles light gray

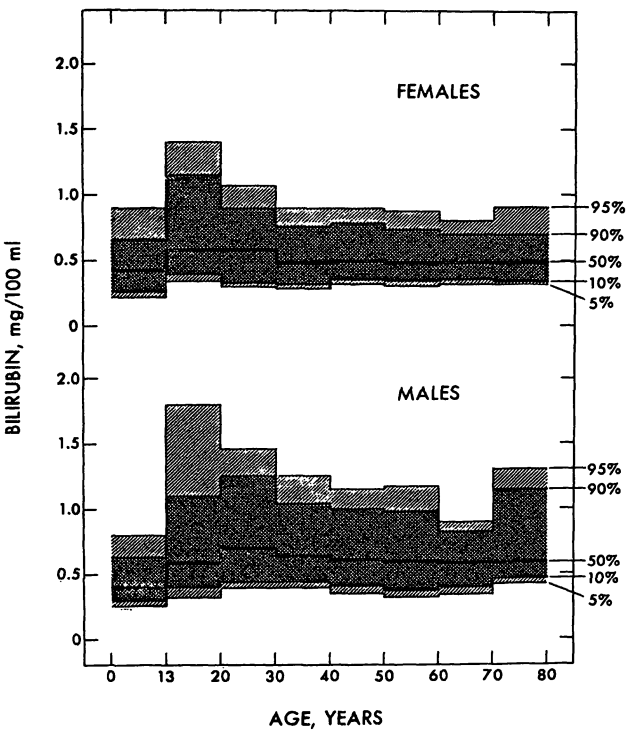

Fig. 11
Serum total bilirubin concentrations in percentiles grouped according to sex and age. The median (50 percentile) is indicated by the heavy line, 10—90 percentiles dark gray, 5—95 percentiles light gray

ated by the Student t-test from the means and standard deviations of the compared groups, since this test is sufficiently flexible to allow for non-normality if the two compared distributions are similar and only a shift in the mean exists (8) (Tables 4 and 5).

Urea nitrogen was higher in males than in females in all age groups, except the eighth decade in which only 27 males were studied (Fig. 8). Concentrations were similar up to the fifth decade; thereafter, both the 50 percentile value and the scatter of results increased.

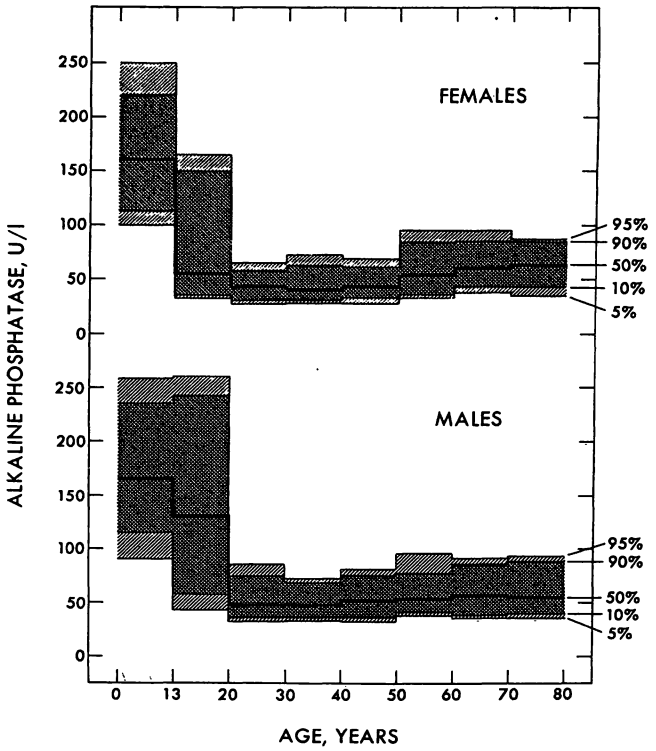

Fig. 12
Serum alkaline phosphatase concentrations in percentiles grouped according to sex and age. The median (50 percentile) is indicated by the heavy line, 10—90 percentiles dark gray, 5—95 percentiles light gray

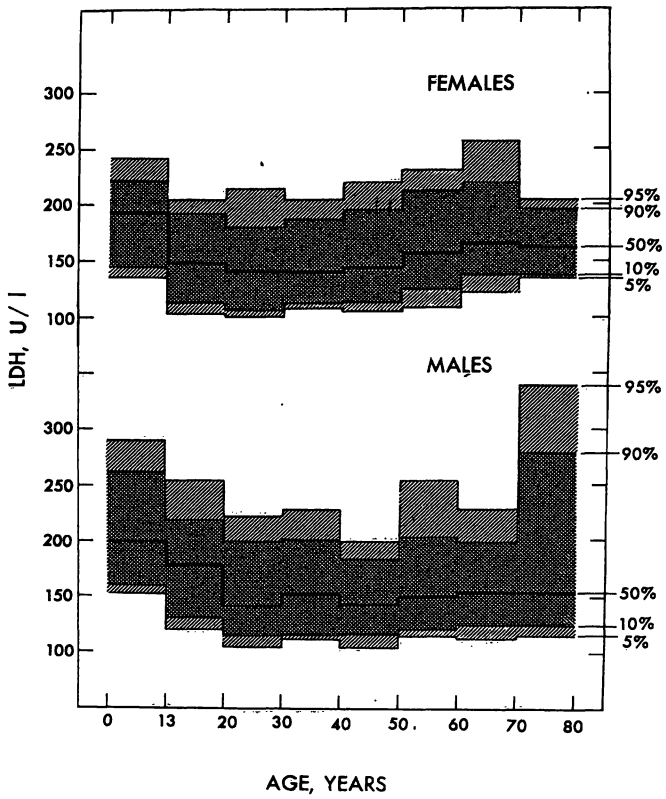

Fig. 13
Serum lactate dehydrogenase concentrations in percentiles grouped according to sex and age. The median (50 percentile) is indicated by the heavy line, 10—90 percentiles dark gray, 5—95 percentiles light gray

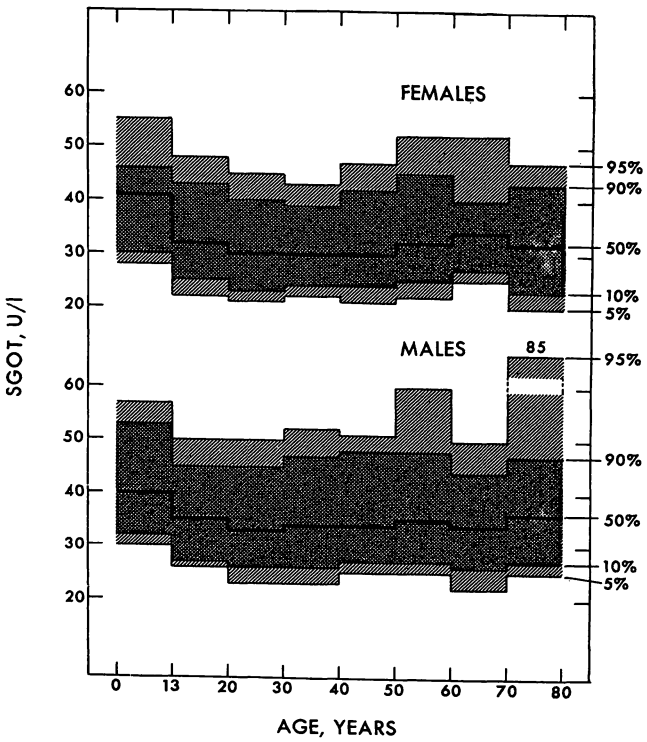

Fig. 14
Serum aspartate transaminase concentrations in percentiles grouped according to sex and age. The median (50 percentile) is indicated by the heavy line, 10—90 percentiles dark gray, 5—95 percentiles light gray

Uric acid in both sexes was similar in childhood; later concentrations were higher in males than in females (Fig. 9). In males uric acid rose sharply at puberty, continued to rise until the third decade, and then was stable with an apparent decrease after age 70. In females there was a lesser rise at puberty, followed by a second, gradual increase after the menopausal age.

Cholesterol rose sharply in the third decade and both sexes had similar values up to that time (Fig. 10). Thereafter, concentrations continued to rise markedly in males until the fifth decade, flattening off later. In females concentrations remained constant until menopause, increasing later to significantly higher values than those of males.

Total bilirubin in both sexes rose after puberty, fell during the third decade, and thereafter remained stable (Fig. 11). After puberty, males had both higher

Tab. 8
Contingency tables relating bilirubin concentrations with lactate dehydrogenase and with aspartate transaminase in the age groups where high bilirubin values are encountered. Numbers of observations are listed. In all four cases the hypothesis of interdependence is rejected by the χ^2-Test ($P > 0.10$)

Males 13 to 30 years		Bilirubin, mg/100 ml 0—0.39	0.40—1.09	1.10—4.00
Lactate Dehydrogenase, U/l	0—109	1	19	3
	110—199	14	164	23
	200—300	0	23	3
Aspartate Transaminase, U/l	0—24.9	3	23	0
	25.0—42.9	10	157	26
	43.0—80.0	1	20	2
Females 13 to 30 years		**Bilirubin, mg/100 ml 0—0.19**	**0.20—0.89**	**0.90—4.00**
Lactate Dehydrogenase, U/l	0—104	2	43	3
	105—179	13	335	37
	180—300	3	20	3
Aspartate Transaminase, U/l	0—20.9	1	27	5
	21.0—37.9	15	311	30
	38.0—80.0	1	48	6

50 percentile values and a more pronounced scatter to high values than females. High bilirubin values during the second and third decade did not correlate with high lactate dehydrogenase, aspartate transaminase, or alkaline phosphatase values (Tab. 8).

Alkaline phosphatase was high in both sexes in the first decade and decreased sharply at puberty (Figs. 12, 15 and 16). Adults showed a slight gradual increase with age. From 20 to 50, values were higher in males than in females; after menopause the increase in females was accelerated, and no sex difference existed.

Lactate dehydrogenase und *aspartate transaminase* behaved similarly (Figs. 13 and 14). After falling at puberty, concentrations in males remained constant and higher than in females. After menopausal age, values in females rose, eliminating the sex difference.

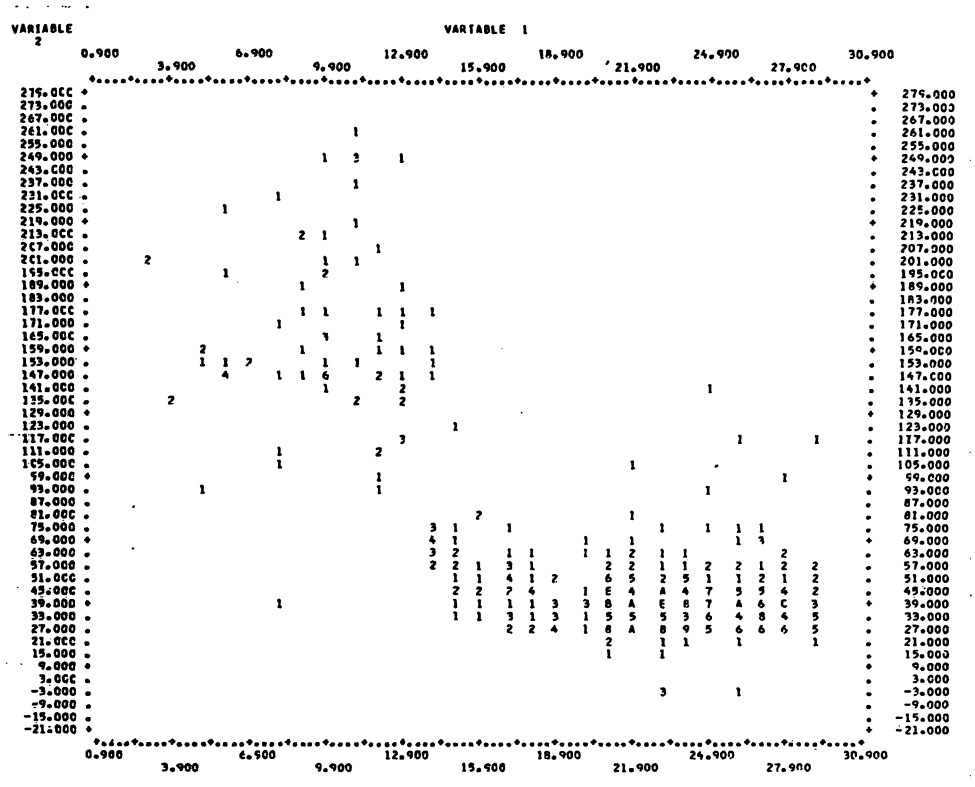

Fig. 15

Correlation of serum alkaline phosphatase concentration (units/liter, y-axis) and age (years, x-axis) for females up to age 30. Numbers indicate observations at any given value. (A = 10, B = 11, ... E = 14). Note distinct fall at puberty

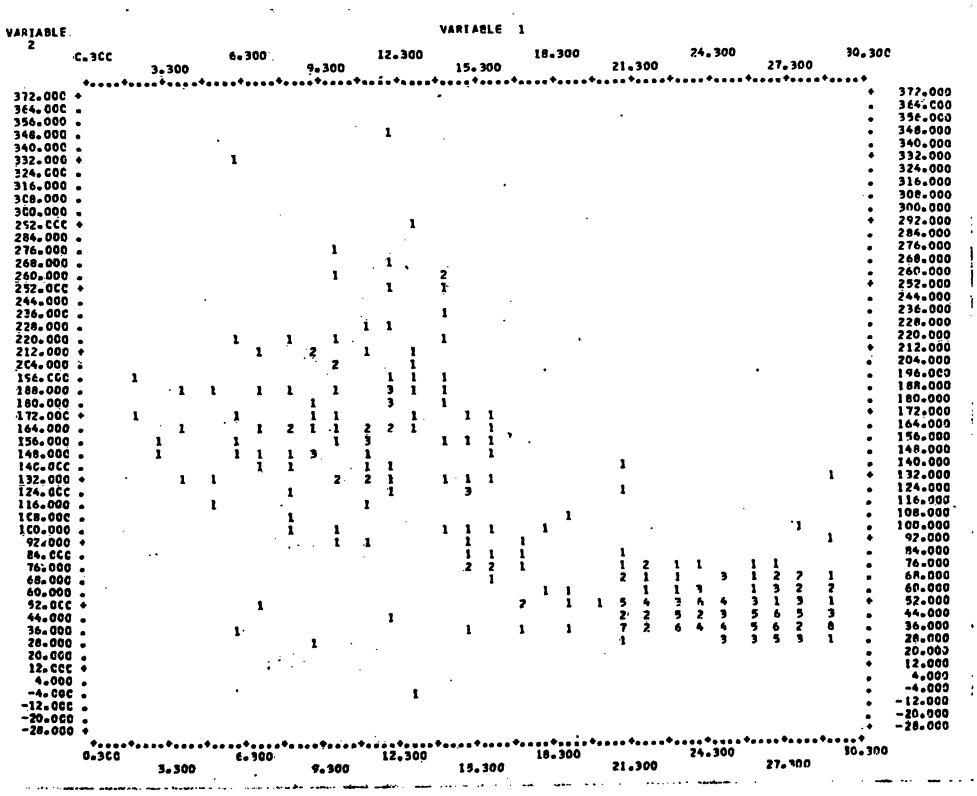

Fig. 16

Correlation of serum alkaline phosphatase concentration (units/liter, y-axis) and age (years, x-axis) for males up to age 30. Numbers indicate observations at any given value

Discussion

Adequate definition together with unbiased selection of a reference population are essential conditions for the establishment of normal limits in medicine. Both definition and selection can be facilitated by increasing the size of the sample. Thus the present study produces almost identical statistics, whether all subjects or only those considered healthy are analyzed. Similarly, a large sample improves definition of normal limits, since it allows grouping of data according to different variables.

All investigated parameters show significant dependence on sex and age which must be taken into account in diagnostic evaluations. The influences of sex and age produce shifts in central values (mean or median) as well as in the scatter of data. The age-sex relationships suggest an endocrine influence by sex hormones (9), since concentrations usually tend to be similar in males and females prior to puberty and again in the seventh and eighth decade, while they differ in the fertile age groups with higher concentrations in males. It is known that the high concentrations of female sex hormones attained during pregnancy lower serum calcium, phosphorus and albumin (10). Considering the effects of aldolescence and menopause, the practice of fitting the dependence of a serum constituent on age to a linear regression equation (5, 11) is questionable. It is also not strictly appropriate to draw conclusions about chronological variations throughout an individual's life from the study of a population cross-section such as this, since there is no assurance that the present group of 70-year-old subjects correctly represents what the 50-year-old subjects will be twenty years later. Thus, only a longitudinal study could reveal whether the observed changes of serum cholesterol concentration after age 70 are due to age or to the selective survival of subjects with lower values.

Sex differences of *calcium* concentration have only been recognized recently with the introduction of newer methods of improved reliability (11—13). The present study shows that these differences act as modifiers in the decrease of serum calcium concentration between childhood and old age. Probably the shifts in total serum calcium are secondary to changes in total protein and albumin concentration, and reflect the homeostatic regulation of ionized calcium, which is thought to be responsible for the physiological activity of this ion. The regression line of serum calcium on total protein concentration extrapolates to 6.25 mg/100 m*l* calcium at zero total protein concentration. In view of the scatter of data, this is in reasonable agreement with directly determined values of ionized serum calcium (14). The extrapolation of the regression line of serum calcium on albumin concentration to 7.95 mg/100 m*l* calcium at zero albumin concentration agrees with the observation that non-ionized calcium is partly bound to other serum proteins than albumin (15). A reported dependence of serum calcium on body height (16) could not be confirmed when the present data were analyzed by sex and age.

Sex differences and age shifts of *total protein* differ from those of *albumin*. In females the rise of total protein at puberty is not accompanied by an increase in albumin. Inhibition of albumin synthesis by both estrogens and progesterone has recently been reported (17). Later in life, shifts in albumin account to a large extent for the changes of total protein in females, while in males the decrease of albumin exceeds that of total protein.

Age and sex dependences of serum *inorganic phosphorus* have been reported in previous studies (11, 18, 19), while others were unable to show them (20). The shifts shown here strikingly resemble those found for *alkaline phosphatase* whose concentration also falls sharply at puberty, is lower in females than in males during the fertile age, and rises again after menopause in females. ALBRIGHT and REIFENSTEIN (21) have pointed out a relationship between the product of serum calcium and inorganic phosphorus concentration and ossification. Since the phosphorus shifts described here are much greater than those of calcium, serum phosphorus largely determines this product, which changes with age and differs between sexes.

Age and sex differences for cholesterol (3, 20, 22) uric acid (20, 23) urea nitrogen (20) and aspartate dehydrogenase (20) have also been reported. If no consideration is given to these effects, unequal representation of subgroups may produce skewed distributions of pooled data as have been described. However, even after sorting for sex and age, skew to high values remains as shown by the unequal distances of the 10 and 90 percentiles from the median (50 percentile) in the cases of *urea nitrogen*, *uric acid*, *cholesterol*, *total bilirubin*, *alkaline phosphatase*, *lactate dehydrogenase* and *aspartate transaminase*. This does not necessarily have to be attributed to the inclusion of pathological values, but may be due to some additional, unrecognized physiological or genetical heterogeneity.

In addition to sex and age, the time after eating, (particularly in the case of phosphorus) and the time of day influence normal values (24). However, subgroups defined according to these time parameters are of similar size in this study, and while they increase scatter, no bias in the distribution of results for calcium, inorganic phosphorus, total protein and albumin is introduced by them, and for the other investigated parameters further grouping of data according to time after eating for a given sex and decade did not remove skew. Since changes in blood constituents relative to the subject's posture have been demonstrated (25), normal values obtained from an ambulatory population may be considered more truly representative for persons seen in the doctor's office.

High *bilirubin* values in the second and third decade, unrelated to high lactate dehydrogenase, aspartate transaminase, or alkaline phosphatase values, have not been previously reported. It is known that GILBERT's

disease manifests itself in the same age groups, and its symptoms decline in later life. Assuming that this syndrome represents a homozygous trait found in about 2% (1/50) of the population (26) about 15% (1/7) of the population would be heterozygous gene carriers ($1/7 \times 1/7 = 1/49$). This prevalence could account for the number of subjects with high bilirubin, and relatives of patients with GILBERT's disease indeed have recently been found to have elevated bilirubin values (27).

Acknowledgments

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References

1. Perkin-Elmer Corp., Calcium in Blood Serum, Tissue and Other Biological Materials. In: Revision of Analytical Methods for Atomic Absorption Spectrophotometry. Norwalk, Conn. (1968). — 2. DAUGHADAY, W. H., M. M. ERICKSON, and W. L. WHITE, Automation in Analytical Chemistry, Technicon Symposia 1967, Vol. 1, p. 91—98. Mediad Inc., White Plains, New York (1968). — 3. HARTMANN, G. and M. WERNER, In: Hyperlipidämien in Klinik und Praxis (Ed. G. Hartmann and F. Wyss) H. Huber, Bern (1970). — 4. HENRY, R. J., Amer. J. Clin. Path. *34*, 226 (1960). — 5. ROBERTS, L. B., Clin. Chim. Acta Amsterdam *16*, 69 (1967). — 6. WOOTTON, I. D. P., and E. J. KING, Lancet, London 1953/I, 470. — 7. HERRERA, L., J. Laborat. Clin. Med. S. Louis *52*, 34 (1958). — 8. GAYEN, E. K., Biometrika, *36* 353 (1949). — 9. FLYNN, F. V., P. GARCIA-WEBB-SHARP, M. J. R. HEALY, K. MACPHERSON, and K. A. PIPER, Enzymol. biol. clin. *10*, 412 (1969). — 10. KERR, C., H. F. LOKEN, M. B. GLENDENING, G. S. GORDAN, and E. W. PAGE, Amer. J. Obstetr. Gynec. *83*, 2 (1962). — 11. KEATING, R. R., Jr., J. D. JONES, L. R. ELVEBACK, and R. V. RANDALL, J. Laborat. Clin. Med. S. Louis *73*, 825 (1969). — 12. JOHNSON, J. R. K. and G. C. RIECHMAN, Clin. Chem. New York *14*, 1218 (1968). — 13. YENDT, E. R. and R. J. A. GAGNE, Canad. Med. Ass. J. *98*, 331 (1968). — 14. FRIZEL, D. E., A. G. MALLESON, and V. MARKS, Clin. Chim. Acta Amsterdam *16*, 45 (1967). — 15. HELD, I. R. and S. FREEMAN. J. Appl. Physiol. *19*, 292 (1964). — 16. ZAPTÁLEK, M., Activitas nervosa superior (Praha) *10*, 251 (1968). — 17. HONGER, P. E. and N. ROSSING, Clin. Sci. *36*, 41 (1969). — 18. BULLOCK, J. K., Amer. J. Dis. Child. *40*, 725 (1930). — 19. GREENBERG, B. G., R. W. WINTERS, and J. B. GRAHAM, J. Clin. Endocr. Springfield *20*, 364 (1960). — 20. ALLERHAND, J., J. MCCARRICK, and L. FISLER, Automation in Analytical Chemistry. Technicon Symposia, 1967, Vol. 1, p. 61—65. Mediad Inc., White Plains, New York (1968). — 21. ALBRIGHT, F. and E. C. REIFENSTEIN, The Parathyroid Glands and Metabolic Bone Diseases, p. 7—17, Williams and Wilkins, Baltimore (1948). — 22. PEZOLD, F. A., Lipide und Lipoproteide im Blutplasma. Biochemie, Pathophysiologie, Klinik. Springer Berlin (1961). — 23. MIKKELSEN, W. M., H. J. DODGE, and H. VALKENBURG, Amer. J. Med. *39*, 242 (1965). — 24. BASSIS, M. L. and M. F. COLLEN, Automation in Analytical Chemistry. Technicon Symposia, 1966, Vol. 1, p. 309—312. Mediad Inc., White Plains, New York (1967). — 25. FAWCETT, J. K. and V. WYNN, J. Clin. Path. London *13*, 304 (1960). — 26. SCHMID, R., Personal Communication. — 27. REDEKER, A. G., D. RICKARD, and B. F. FELSCHER, Gastroenterology, in press.

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